HIV-1 interaction with the nuclear pore complex

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1. BACKGROUND: HIV-1 replication cycle

ABSTRACT. Human immunodeficiency virus remains a major global health issue, with over 1.3 million people newly infected in 2022 alone. HIV is a virus that attacks vital cells in humans, weakening the immune system. While HIV has been studied for many decades, there are still gaps in this area of research. HIV infection of cells depends on its ability to enter the nucleus of non-dividing cells (Fig. 1A) and integrate a copy of its DNA into host cells. How HIV passes through the nuclear pore complex remains unclear (Fig. 1B). In this project, we look at HIV-1 interaction with the nuclear pore complex, which depends on the interactions between the viral capsid protein and nuclear pore-associated NUP358, NUP153 proteins. During my UROP project, I performed image analysis to determine the ability of HIV capsid to dock at the nuclear pore. Fluorescent imaging and processing of HIV-1 infected cells were conducted using ICY bioimage analysis. The results show that our method for docking determination works well and can report the block of HIV-NPC interactions by small molecule inhibitors PF74 and LEN. Future studies are being conducted to understand HIV nuclear entry and to develop additional inhibitors targeting this crucial step.

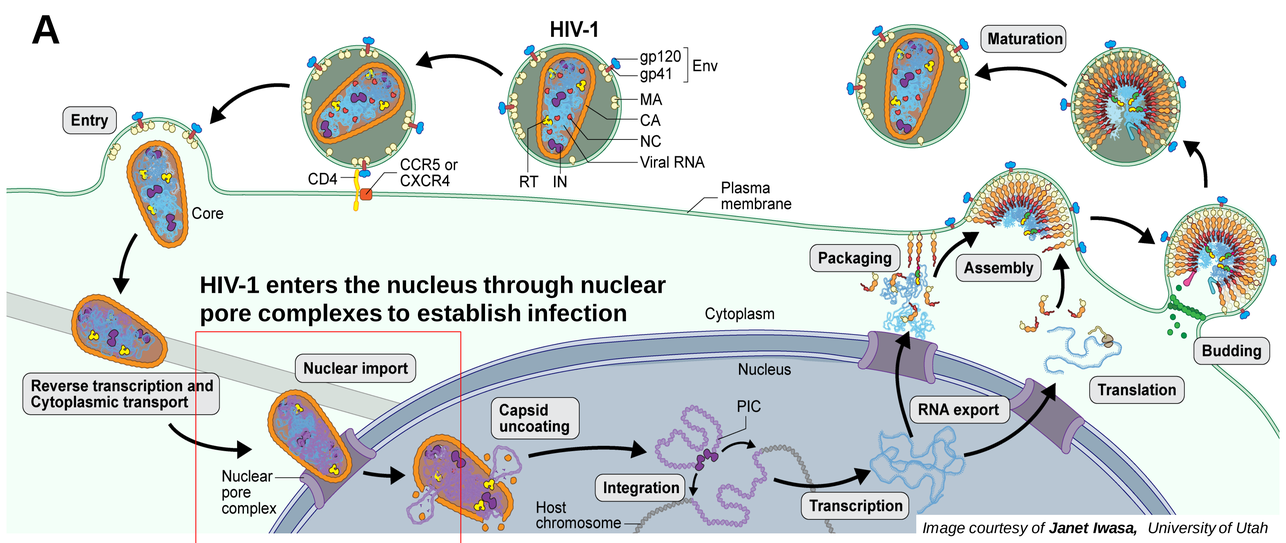

Fig-1. (A) The life cycle of HIV-1 progresses through distinct steps. **(B)** HIV-1 nuclear import proceeds through a series of crucial steps. (***i***) First, the capsid must engage and stabilize interactions with the nuclear pore complex (NPC) through the step of 'docking', (***ii***) next, the capsid percolates into the central NPC channel, and (***iii***) finally, competitive interactions between CPSF6 and NUP153 releases the capsid into the nucleus**.** *Taken from Muller et. al., Annu. Rev. Virol. 2022*

2. METHOD: Tracking HIV-1 NPC interactions in living cells by confocal microscopy

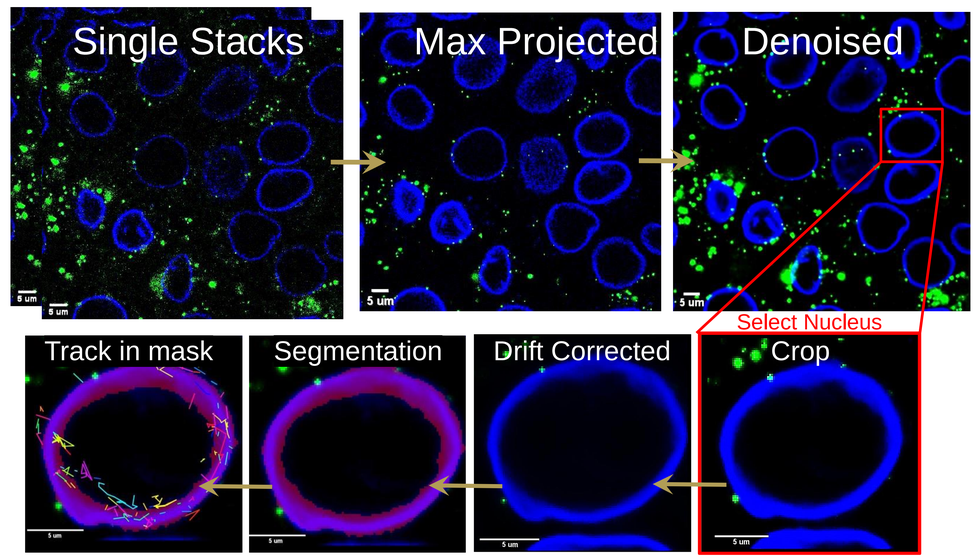

Fig-2. The 3D time-lapse imaging of HIV-1 infected TZM-bl cells was carried out for 16 hours by acquiring 9-11 Z-stacks spaced by 0.8 µm, for every 2.5 min. The central z-stacks (1.6 um) is extracted from the 3D datasets and projected onto a 2D image. Single nuclei are cropped from the denoised images, and drift corrected by then used for final tracking. Segmentation of the lamin nuclear envelope marker generates a region of interest (ROI) mask (*bottom, middle panel*), and HIV-1 particles that coincide with this mask are detected and tracked in an automated fashion on the ICY bioimage analysis software. The bottom left most image shows example tracks overlaid on the lamin mask. Single HIV-1 particles are labeled with INmNG vRNP marker. The nuclear envelope in target TZMbl cells are labeled with emiRFP670-laminB1.

3. RESULTS: Analysis of HIV-NPC interactions

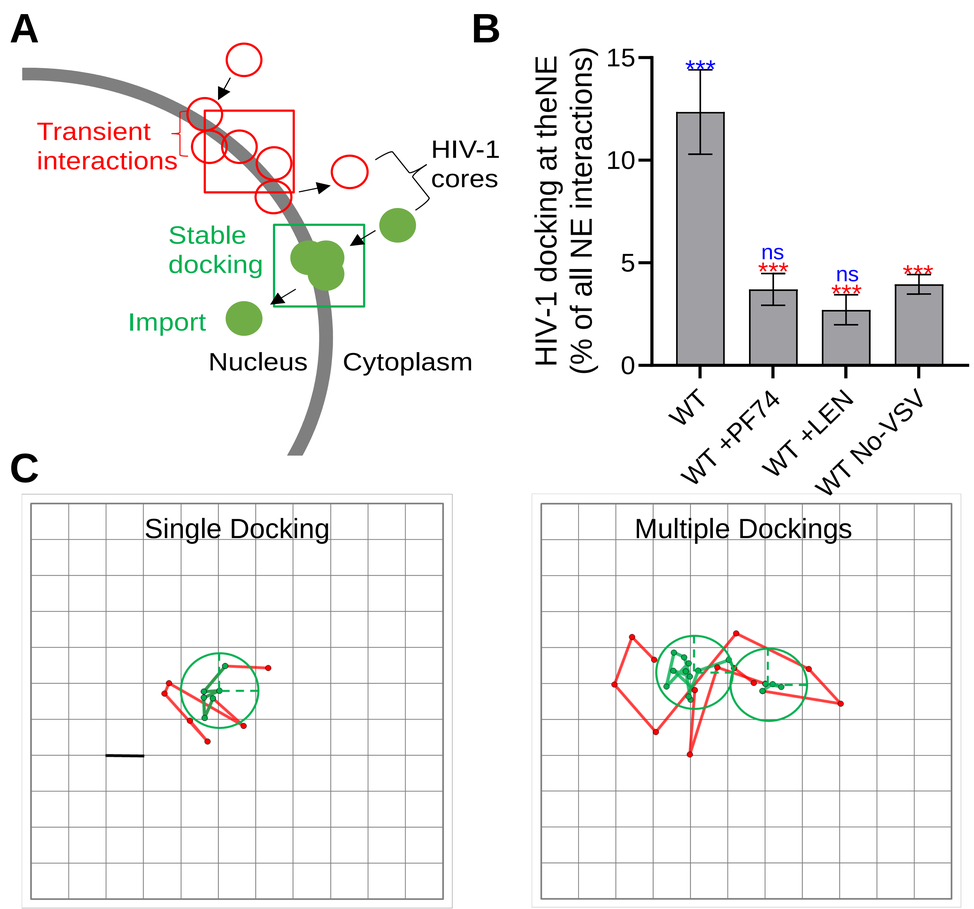

Fig.3 – (A) Cartoon depicting multiple modes of HIV-1 interaction with the nuclear membrane (*as determined by automated tracking Fig. 2D).* Transient interactions (a track showing motion), and stable interactions (tracks that remain localized within 360 nm radius for 3 or more frames). **(B)** The fraction of stable interactions (docking, % of total tracks) was determined for HIV-1 (WT), or controls HIV+ 2uM PF74 or 500nM LEN which block HIV nuclear import. The background docking was detected by tracking endosome residing bald HIV-1 core (no-VSV-G). Data shows a clear distinction of docking only for WT infections. **(C)** Example trajectories showing one (left) and multiple docking profiles in single trajectories.

4. SUMMARY

Our research has demonstrated that our method of docking determination can report the block of HIV-NPC interactions by small molecule inhibitors PF74 and LEN. Tracking of HIV-1 NPC interactions in living cells was conducted through 3D time lapse imaging and analyzed on ICY bioimage analysis software. Data analysis of the HIV-NPC interactions shows clear distinction of docking for only WT infections. Future direction will look at HIV nuclear entry and aim to develop additional inhibitors to target this crucial step.

5. REFERENCES

Muller TG, et.al, Nuclear Capsid Uncoating and Reverse Transcription of HIV-1. Annu Rev Virol. 2022 Sep 29;9(1):261-284. PMID:35704745

6. ACKNOWLEDGEMENTS

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